INFECTION AND IMMUNITY, July 1988, p. 1685–1691
0019-9567/88/071685-07$02.00/0

Vol. 56, No. 7

Role of L3T4$^+$ T Cells in Host Defense against *Histoplasma capsulatum*

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Received 21 December 1987/Accepted 11 March 1988

Cell-mediated immunity is critical in host resistance against the pathogenic fungus *Histoplasma capsulatum*. To explore the role of L3T4$^+$ T cells in protection of mice against *H. capsulatum* infection, we examined the effect of in vivo treatment with anti-L3T4 monoclonal antibody (MAb) GK1.5 on the course of murine disseminated histoplasmosis. Treatment with anti-L3T4 antibody caused a profound and selective depletion of L3T4$^+$ T cells that was associated with a significant increase in the number of *H. capsulatum* CFU recovered from the spleens of mice infected for 1 week. In addition, none of the infected mice treated with MAb GK1.5 survived a sublethal challenge with *H. capsulatum* yeasts. Histopathological examination of spleens from mice infected for 1 week revealed the presence of granulomatous inflammation in mice depleted of L3T4$^+$ T cells and in infected controls. However, silver stains demonstrated that spleens of infected mice given MAb GK1.5 contained a greater number of yeasts than did spleens from infected controls. MAb GK1.5 did not cause reactivation of infection when administered for 2 weeks beginning 4 weeks after inoculation of *Histoplasma* yeasts. MAb GK1.5 did not alter the functional properties of murine macrophages as measured by antigen presentation, production of interleukin-1 in response to lipopolysaccharide, and phagocytosis of *H. capsulatum* yeasts. These results suggest that the L3T4$^+$ T-cell subset is an essential constituent of the cell-mediated immune defense against *H. capsulatum* infection.

An effective host response that controls infection with the dimorphic fungus *Histoplasma capsulatum* requires activation of cell-mediated immune responses (1, 2, 5, 13, 17). Recent evidence suggests that L3T4$^+$ T cells are important constituents of host defenses against infection with this pathogenic organism (5). *H. capsulatum*-reactive murine T-cell clones that are L3T4$^+$ mediate local delayed-type hypersensitivity responses to histoplasmin (HKC) when injected with antigen into the footpads of naive mice. Moreover, after stimulation with HKC, these cells secrete interleukin-2 (IL-2) and a factor resembling gamma interferon that arms macrophages to inhibit intracellular growth of *H. capsulatum* yeasts (5). However, little is known about the in vivo role of the L3T4$^+$ T-cell subpopulation in host resistance to *H. capsulatum*. Therefore, we examined the effect of monoclonal antibody (MAb) GK1.5 in C57BL/6 mice systemically infected with *H. capsulatum*. In vivo injection of this MAb profoundly reduced the number of L3T4$^+$ T cells in lymphoid organs of mice infected for 1 week. Administration of MAb GK1.5 to infected mice for 1 week was associated with a significant increase in the number of *H. capsulatum* CFU recovered from spleens of mice compared with infected controls. In survival studies, 100% of MAb-treated animals died by day 14, whereas all control infected mice survived beyond day 21. Histopathologically, a granulomatous inflammatory response was present in the spleens of both control infected mice and those depleted of L3T4$^+$ cells.

MATERIALS AND METHODS

Mice. Male C57BL/6 and C3H/HeJ mice were purchased from Jackson Laboratory (Bar Harbor, Maine).

Infection with *H. capsulatum*. Preparation and inoculation of *H. capsulatum* yeasts (strain G217-B) has been described previously (2). Mice 6 to 8 weeks old were inoculated via the tail vein with 6×10^5 yeasts suspended in 0.2 ml of Hanks balanced salt solution. This is a sublethal inoculum that induces less than 5% mortality in mice 30 days postinoculation (2). Normal mice housed under identical conditions were used as age-matched controls.

Antibody treatment. The GK1.5 cell line producing anti-L3T4 MAb was obtained from the American Type Culture Collection (Rockville, Md.). Cells (10^5/ml) were inoculated into serum-free RPMI 1640 medium containing 1% nonessential amino acids, 1% L-glutamine, 1% sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol, 10 μg of gentamicin per ml, and 1% Nutridoma NS (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and cultured for 6 days at 37°C in a 5% CO_2 atmosphere. Subsequently, cells were removed by centrifugation, and the supernatant was concentrated by ultrafiltration through a PM10 membrane (Amicon Corp., Danver, Mass.) to a volume approximately 1/15th of the original. This was dialyzed against phosphate-buffered saline (pH 7.0) overnight at 4°C, filter sterilized, and stored at −20°C until used. The concentration of rat immunoglobulin G (IgG) in supernatants was assessed by a solid-phase enzyme-linked immunosorbent assay and calculated by linear regression from a rat IgG standard curve.

Beginning 4 h after intravenous (i.v.) inoculation of yeasts, mice were injected intraperitoneally (i.p.) with 20 μg of MAb GK1.5 per day for 1 week. Control mice were given an equal volume of saline rather than a rat IgG because repeated administration of the latter caused anaphylaxis and death. In pretreatment experiments, MAb (20 μg/day) or saline administration was initiated 1 week before infection and continued daily for an additional week thereafter.

Antigens and mitogens. Sheep erythrocytes were purchased from Colorado Serum Co. (Denver, Colo.). HKC was prepared from six mycelial strains of *H. capsulatum* grown in Smith asparagine medium. Before use, HKC was

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dialyzed extensively against phosphate-buffered saline (pH 7.0). Concanavalin A (grade IV) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Phytohemagglutinin was obtained from Burroughs Wellcome Co. (Research Triangle Park, N.C.).

Cell preparation. Spleens and inguinal, mesenteric, and paraortic lymph nodes were teased apart between the frosted ends of two ground glass slides, washed twice in Hanks balanced salt solution, and resuspended in medium. A population of splenocytes enriched for T cells was prepared by the nylon wool column method of Julius et al. (7).

Fluorescence analysis. Splenocytes and lymph node cells were suspended in RPMI 1640 medium containing 1% fetal bovine serum and 0.1% sodium azide (staining medium). To 10^6 cells in 200 μl was added 7.5 μl of fluorescein-conjugated anti-Thy-1.2 MAb or anti-Lyt-2 MAb (Becton Dickinson and Co., Mountain View, Calif.). To label B cells, 50 μl of $F(ab')_2$ fragment of fluoresceinated goat anti-mouse IgG (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md.) was added to a separate lot of cells at a 1:20 dilution of a stock solution. Cells were incubated at 4°C for 30 min and then washed twice in staining medium. To label $L3T4^+$ cells, splenocytes and lymph node cells were incubated with 200 μl of supernatant from hybridoma GK1.5 for 30 min at 4°C followed by the addition of 150 μl of a 1:40 dilution of fluorescein-conjugated goat anti-rat IgG absorbed with mouse serum (Kirkegaard and Perry Laboratories). All cells were suspended in 500 μl of staining medium before analysis with a fluorescence-activated cell sorter (FACS 440; Becton Dickinson). In addition, a sample of cells from each organ was cytocentrifuged and stained with Diff-Quik (Dade Diagnostics, Inc., Aquada, P.R.).

Organ culture of *H. capsulatum* and histopathology. Cultures of spleens for determination of *H. capsulatum* CFU were performed as reported previously (6). Tissue specimens from spleens were fixed in 10% buffered Formalin, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin and methenamine-silver (Grocott).

Mitogenic and antigen stimulation assay. Responses to concanavalin A were measured as described previously (6). Results are expressed as mean counts per minute of triplicate cultures.

In the antigen proliferation assay, three *H. capsulatum*-reactive T-cell clones (2.3H3, 2.3E10, and 1.3G6) and an *H. capsulatum*-reactive T-cell line (JC1) were used (5). Resting cloned T cells were suspended in RPMI 1640 containing 10% fetal bovine serum and 10 μg of gentamicin per ml at a concentration of 2×10^5 cells per ml. To each well of a microtiter plate were added 2×10^4 T cells in 0.1 ml. As a source of antigen-presenting cells, irradiated splenocytes (1,500 rads) from syngeneic mice were used. To each well were added 5×10^5 irradiated splenocytes in 0.1 ml and 50 μl of HKC. Cells were incubated for 72 h at 37°C in a 5% CO_2 atmosphere; 16 h before harvest, 0.5 μCi of [^{3}H]thymidine was added to each culture.

In vitro antibody production. Spleen cells were cultured as described by Mishell and Dutton (10); 1.5×10^7 cells were dispensed into each well of a six-well culture plate (35-mm diameter) and immunized with 3.5×10^6 sheep erythrocytes. Plates were incubated at 37°C for 4 days in 5% CO_2. The IgM antibody plaque-forming cells (PFC) were assessed by the method of Cunningham and Szenberg (4). In coculture experiments, 5×10^6 cells from normal or infected mice were added to 1×10^7 normal splenocytes. The data are calculated by dividing the number of PFC per culture over the number of viable cells recovered after 4 days of culture. The mean number of PFC per 10^6 cells recovered was calculated from the results of quadruplicate cultures.

Generation of IL-1 containing supernatants. IL-1-containing supernatants from adherent splenocytes were prepared as reported previously (15). To quantitate the number of macrophages per well, we removed adherent splenocytes from three or four plates by scraping with a rubber policeman. In addition, 25-mm round cover slips were placed in wells and stained with nonspecific esterase (Sigma). The absolute number of macrophages was determined by multiplying the percentage of esterase-positive cells by the number of recovered cells.

Assay for IL-1 activity. To 0.1 ml of twofold dilutions of test supernatants were added 10^6 thymocytes from C3H/HeJ mice in 0.1 ml of RPMI 1640 containing 10% fetal bovine serum and 10 μg of gentamicin per ml. Triplicate cultures were incubated at 37°C in a 5% CO_2 atmosphere for 72 h in the presence or absence of 1 μg of phytohemagglutinin per ml. Sixteen hours before harvesting, cells were pulsed with 0.5 μCi of [^{3}H]thymidine. IL-1 units were calculated by the equation: (counts per minute of the test supernatant at 1:2 dilution/counts per minute of thymocytes incubated with a suboptimal concentration of phytohemagglutinin) − 1.

Intracellular proliferation of *H. capsulatum*. Intracellular growth of *H. capsulatum* was assayed by the method of Wu-Hsieh and Howard (19). Peritoneal macrophages were harvested with RPMI 1640 containing 10 U of heparin per ml from mice that had been inoculated i.p. with 2 ml of Proteose Peptone (Difco Laboratories, Detroit, Mich.) 2 days previously. Macrophages were washed and resuspended in RPMI 1640 containing 10% fetal bovine serum and 10 μg of gentamicin per ml to a final concentration of 10^6 cells per ml; 1-ml samples were placed on 25-mm round cover slips in 35-mm plastic dishes and incubated for 2 h at 37°C. The dishes were then washed extensively, and the monolayers were inoculated with 2×10^5 *Histoplasma* yeast cells. Extracellular yeasts were removed after 1 h, and cover slips from each group were removed and stained with Diff-Quik. The remaining monolayers were incubated for an additional 15 h before staining. The number of yeasts within 100 infected macrophages was counted, and the mean number of yeast cells per macrophage was calculated.

Statistical analysis. The Wilcoxon rank sum test was used for comparison of two groups. Fischer's exact test was used for comparison of two proportions.

RESULTS

Effect of MAb GK1.5 treatment on lymphocyte populations in *H. capsulatum*-infected mice. MAb GK1.5 or saline was administered i.p. beginning 4 h after i.v. injection of 6×10^5 *H. capsulatum* yeasts, and treatment was continued daily for 1 week. Subsequently, the phenotype of lymphocyte populations in spleens and lymph nodes of individual mice was determined by cytofluorometric analysis. There was a significant reduction ($P < 0.01$) in both the number and percentage of $Thy\text{-}1.2^+$ and $L3T4^+$ cells in spleens and lymph nodes of anti-L3T4-treated mice as compared with saline recipients (Table 1). The depletion of $L3T4^+$ cells was >90% in each organ and was selective since the number of $Lyt\text{-}2^+$ cells and B cells did not differ significantly between the MAb-treated and control groups.

***H. capsulatum* CFU in spleens of mice treated with MAb GK1.5.** Mice were injected i.p. daily with 20 μg of MAb GK1.5 or saline for 1 week and then inoculated with 6×10^5

TABLE 1. Surface phenotype of lymphoid cells from *H. capsulatum*-infected mice treated with MAb GK1.5

Organ	Lymphocytes	Mean no. of cells (10^5)[a]	
		Saline	MAb GK1.5
Spleen	Total	87.2 ± 11.8	65.9 ± 9.0
	Thy-1.2+	20.9 ± 3.1 (24)	8.3 ± 1.2 (13)
	L3T4+	10.3 ± 1.5 (12)	0.7 ± 0.2 (1)
	Lyt-2+	8.9 ± 1.4 (10)	8.3 ± 1.3 (13)
	B	59.3 ± 7.0 (71)	47.2 ± 6.5 (71)
Lymph nodes	Total	7.7 ± 0.3	4.8 ± 0.3
	Thy-1.2+	4.8 ± 0.4 (63)	2.5 ± 0.2 (51)
	L3T4+	2.8 ± 0.2 (37)	0.12 ± 0.07 (3)
	Lyt-2+	1.7 ± 0.3 (22)	2.1 ± 0.1 (45)
	B	2.8 ± 0.2 (37)	2.4 ± 0.4 (48)

[a] Mean ± SEM of five individual mice. Figures in parentheses indicate mean percentage of marker-positive lymphocytes.

yeast cells. Administration of MAb GK1.5 was continued daily for another week. In three experiments, pretreatment with MAb GK1.5 increased the number of *H. capsulatum* CFU in spleens by 58 to 256% (Table 2).

Additional experiments were performed to determine whether depletion of L3T4+ cells after a challenge with *H. capsulatum* would also modify infection. Beginning 4 h after i.v. injection of yeasts, groups of mice were injected i.p. with 20 μg of MAb GK1.5 or saline for 1 week. The number of *H. capsulatum* CFU recovered from spleens of mice treated daily with 20 μg of MAb GK1.5 was greater ($P < 0.01$) than that cultured from spleens of saline recipients (Fig. 1). Spleens from MAb-treated mice in two experiments contained 156 and 200% more CFU, respectively, than spleens from controls. The mean weight of the spleens from infected MAb-treated mice (0.284 ± 0.02 g) was slightly less ($P > 0.05$) than that of spleens of saline recipients (0.317 ± 0.04 g). Therefore, the increase in number of *H. capsulatum* CFU recovered from spleens of MAb GK1.5 recipients cannot be explained by organomegaly. These results indicate that elimination of L3T4+ cells either before or after inoculation of *Histoplasma* yeasts produced an exacerbation of infection. Furthermore, the increase in severity of infection in mice depleted of L3T4+ cells after establishment of infection was similar to that of animals in whom these cells were eliminated by pretreatment.

Histopathology. Histopathological examination revealed the presence of granulomatous inflammation in the spleens of infected mice given either MAb GK1.5 or saline (Fig. 2A and B). In both groups, the red pulp and the periarteriolar lymphocyte sheaths and marginal zones of the white pulp were infiltrated heavily by macrophages. In addition, disruption of malpighian corpuscles was detected in specimens from MAb GK1.5-treated animals and infected controls. Treatment with anti-L3T4 antibody did not induce granulomatous inflammation in spleens of normal mice. Silver stains of specimens demonstrated yeast cells scattered throughout the red and white pulp (Fig. 2C and D). However, the number of yeast cells in sections of spleens from mice administered MAb GK1.5 (93 ± 7 per high-power field) was significantly greater ($P < 0.01$) than that observed in saline recipients (26 ± 4 per high-power field).

TABLE 2. Effect of pretreatment with MAb GK1.5 on the number of CFU recovered from spleens of *H. capsulatum*-infected mice

Treatment[a]	Mean CFU/spleen (10^6)		
	Expt 1	Expt 2	Expt 3
Saline	0.9 ± 0.1	0.7 ± 0.6	0.8 ± 0.2
MAb GK1.5	3.2 ± 0.5[b]	1.1 ± 0.5[c]	1.7 ± 0.3[c]

[a] Mice were treated with MAb GK 1.5 (20 μg/day) or saline for 1 week and then inoculated with 6×10^5 *H. capsulatum* yeasts. Treatment was continued daily for another week. At week 1 of infection, spleens were removed and cultured.

[b] $P = 0.01$ compared with control group.

[c] $P = 0.05$ compared with control group.

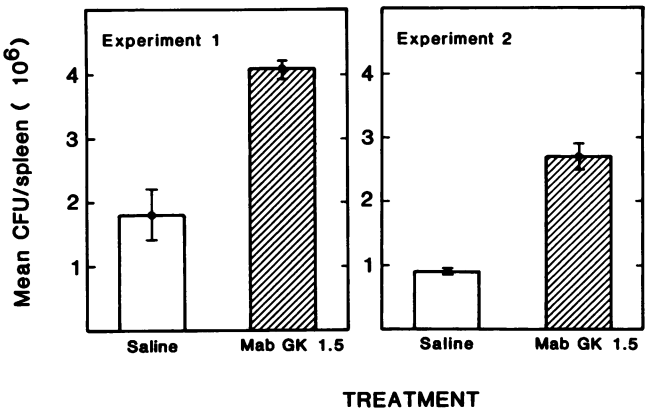

FIG. 1. Effect of MAb GK1.5 on the number of CFU recovered from spleens of *H. capsulatum*-infected mice. Groups of five mice were inoculated with 6×10^5 *H. capsulatum* yeast cells i.v. and then given either saline i.p. or MAb GK1.5 (20 μg i.p. per day) for 1 week. At that time, spleens were removed and cultured.

Survival of *H. capsulatum*-infected mice treated with MAb GK1.5. To ascertain whether administration of MAb GK1.5 altered the course of disseminated histoplasmosis, groups of 10 mice were infected and treated with either MAb GK1.5 or saline for 1 week. Mice were observed daily, and the survival was recorded for 21 days. Signs of infection (inactivity, weight loss, and ruffled fur) appeared around day 7 in both groups but were more severe and rapidly progressive in the anti-L3T4-treated mice. By day 9 all MAb recipients were huddling and inactive, and by day 14 100% of these mice were dead (Fig. 3).

Reaction of *Histoplasma* infection by MAb GK1.5. To investigate whether treatment with MAb GK1.5 could reactivate disease during recovery from systemic infection, we gave 20 μg of antibody or saline i.p. for 2 weeks to mice that had been inoculated with *H. capsulatum* 4 weeks previously. Mice were then sacrificed, and the number of CFU in the spleens was determined. In three experiments, low numbers (<100 CFU) were recovered from the spleens of 6 of 14 antibody-treated mice and from the spleens of 2 of 15 saline recipients ($P > 0.05$). Under these conditions, MAb GK1.5 did not appear to induce reactivation of *Histoplasma* infection.

Alteration of in vitro cellular immune responses of infected mice by MAb GK1.5. In a representative experiment, the mean proliferative response (± standard error of the mean [SEM]) to concanavalin A by splenocytes from normal mice given MAb GK1.5 was markedly reduced (15,983 ± 543 cpm; medium control, 2,470 ± 12 cpm) compared with the response of splenocytes from saline-treated mice (73,937 ± 1,291 cpm; medium control, 1,365 ± 87 cpm). The response to concanavalin A by splenocytes from infected mice at week 1 (5,709 ± 38 cpm; medium control, 104 ± 21 cpm) was considerably depressed compared with that of cells from

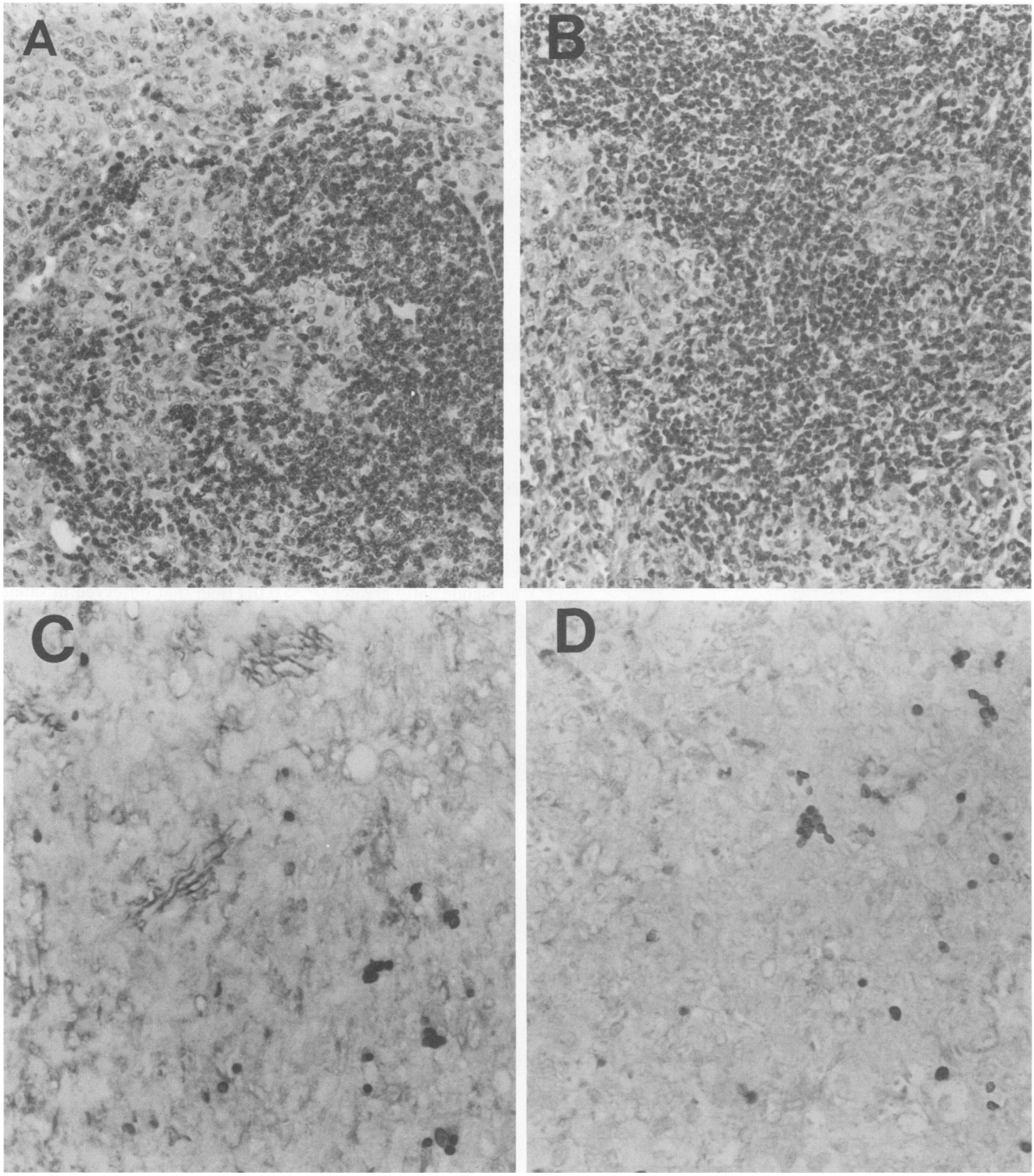

FIG. 2. Photomicrographs of spleens from mice infected for 1 week and given either saline or MAb GK1.5. (A) Splenic white and red pulp from a saline recipient invaded by pale-staining macrophages (hematoxylin and eosin, ×100). (C) Grocott stain of the same spleen as in panel A (×400). (B) Splenic white and red pulp from infected mouse treated with MAb GK1.5. There is granulomatous inflammation and disruption of the malpighian corpuscles (hematoxylin and eosin, ×100). (D) Silver stain of the same spleen as in panel B. A large number of yeasts are present (×400).

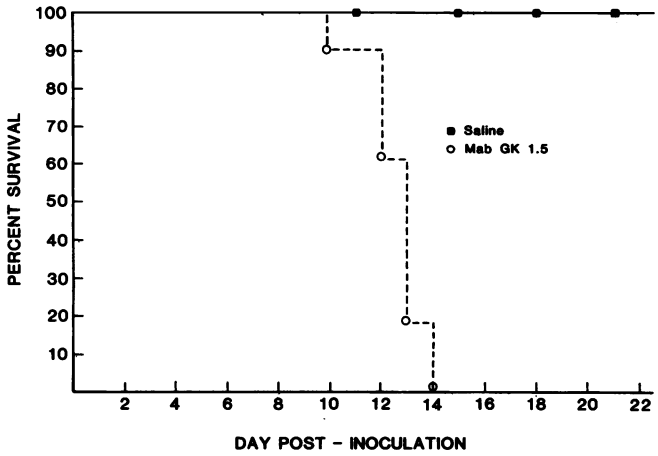

FIG. 3. Effect of MAb GK1.5 on the survival of *H. capsulatum*-infected mice. On day 0, groups of 10 mice were inoculated i.v. with 6×10^5 *H. capsulatum* yeast cells and then given either saline or MAb GK1.5 (20 μg/day) for 1 week. Treatment was stopped on day 7, and mice were observed through day 21.

normal mice as a consequence of suppressor cell activity previously described by members of our laboratory (2). The response by splenocytes from infected mice treated with MAb (1,375 ± 227; medium control, 163 ± 37 cpm) was even less than that of infected controls.

Additional experiments were performed to examine the influence of MAb GK1.5 on the in vitro suppressor cell activity exerted by splenocytes at 1 week of infection (2, 12). After i.v. inoculation of *H. capsulatum*, mice were given 20 μg of MAb GK1.5 i.p. per day for 7 days. Concomitantly, MAb was given to normal mice. The PFC response by normal spleen cells cocultured with splenocytes from normal mice administered MAb GK1.5 did not differ significantly ($P > 0.05$) from that of spleen cells from untreated mice (Table 3). Unfractionated splenocytes from infected mice given either MAb GK1.5 or saline markedly depressed the PFC response by normal syngeneic spleen cells (Table 3). In addition, suppressor activity of splenic T cells (nylon wool passed) or macrophagelike splenocytes (nylon wool adherent) from MAb GK1.5-treated mice did not differ ($P > 0.05$) from that of infected controls (Table 3). Therefore, treatment with anti-L3T4 antibody did not modify the suppression

TABLE 4. Antigen-presenting function of spleen cells from mice treated with MAb GK1.5

T-cell line or clone	HKC[a]	Spleen cell source of APC[b]	
		Saline[c]	MAb GK1.5[c]
2.3H3	−	314 ± 24	286 ± 6
	+	6,584 ± 282	8,153 ± 224
2.3E10	−	323 ± 40	355 ± 38
	+	7,898 ± 602	7,691 ± 19
1.3G6	−	454 ± 37	452 ± 42
	+	7,076 ± 182	9,937 ± 412
JC1	−	1,031 ± 54	1,328 ± 137
	+	13,570 ± 411	15,227 ± 1,618

[a] Final dilution of HKC in wells was 1:100 for 2.3E10 and 1:500 for 2.3H3, 1.3G6, and JC1.
[b] APC, Antigen-presenting cell. Mean counts per minute ± SEM of triplicate determinations.
[c] Mice treated with MAb GK 1.5 (20 μg/day) or saline for 1 week.

exerted by unfractionated cells or by either fraction of splenocytes from infected mice.

Effect of MAb GK1.5 on macrophage function in normal mice. Human and rat macrophages bear molecules that are homologous to the murine L3T4 antigen (8). Since the principal effector cell in host resistance to *H. capsulatum* is the macrophage, several studies were conducted to determine whether MAb GK1.5 modified certain functional properties of normal murine macrophages. First, we assessed the proliferative response by three *H. capsulatum*-reactive T-cell clones, 2.3H3, 2.3E10, and 1.3G6, and one *H. capsulatum*-reactive T-cell line, JC1, to HKC in the presence of irradiated splenocytes (as source of antigen-presenting cells) from normal mice given either saline or MAb GK1.5. The cloned T cells and the T-cell line responded equally well to HKC when cultured with either preparation of antigen-presenting cells (Table 4).

In a second set of studies, supernatants of lipopolysaccharide-stimulated adherent splenocytes from mice that were administered either saline or MAb GK1.5 (20 μg daily for 1 week) were tested for IL-1 activity. Production of IL-1 by adherent splenocytes from mice given anti-L3T4 MAb was similar to that of adherent spleen cells from control mice (Table 5).

Finally, we determined whether anti-L3T4 antibody altered intracellular growth or phagocytosis of *H. capsulatum* by peritoneal macrophages. Proteose-Peptone-elicited peri-

TABLE 3. Suppressor cell activity in spleens of *H. capsulatum*-infected mice treated with MAb GK1.5

Splenocytes added to normal spleen cells[a]	Splenocyte donor	Treatment with MAb GK1.5[b]	PFC/10^6 recovered cells ± SEM[c]	
			Expt 1	Expt 2
Unfractionated	Normal	−	2,262 ± 210	3,378 ± 191
		+	2,085 ± 148 (92)	3,064 ± 127 (90)
	Infected	−	475 ± 195 (21)	142 ± 12 (4)
		+	228 ± 50 (10)	238 ± 30 (7)
Nylon wool passed	Normal	−	2,914 ± 150 (128)	3,854 ± 137 (114)
		+	3,017 ± 273 (133)	2,999 ± 286 (88)
	Infected	−	1,053 ± 155 (47)	1,306 ± 117 (39)
		+	1,089 ± 202 (48)	1,263 ± 126 (37)
Nylon wool adherent	Normal	−	2,284 ± 196 (100)	3,538 ± 137 (104)
		+	2,628 ± 280 (116)	3,339 ± 127 (99)
	Infected	−	253 ± 34 (11)	174 ± 23 (5)
		+	268 ± 19 (12)	126 ± 20 (4)

[a] 5×10^6 spleen cells from normal or infected mice added to 1×10^7 normal splenocytes.
[b] Mice received 20 μg of MAb GK1.5 or saline i.p. every day for 7 days.
[c] Figures in parentheses indicate percent control response.

TABLE 5. Production of IL-1 by adherent spleen cells from mice treated with MAb GK1.5

Expt	Treatment with MAb GK1.5[a]	IL-1 units/10^5 adherent cells in response to:	
		Medium	LPS[b]
1	−	1.4	3.9
	+	1.7	5.6
2	−	1.6	7.1
	+	1.6	5.5
3	−	1.2	3.2
	+	2.0	6.5

[a] Mice were treated for 1 week with 20 μg of MAb GK1.5 per day or saline.
[b] Final concentration of lipopolysaccharide (LPS) was 50 μg/ml.

toneal macrophages were harvested from treated and untreated mice and infected with *H. capsulatum* yeasts for 15 h. In two experiments, the mean number (± SEM) of intracellular yeasts per infected macrophage from mice given MAb GK1.5 increased from 2.6 ± 0.2 to 6.9 ± 0.4 at 15 h; the number of yeasts per infected macrophage from control mice increased from 2.1 ± 0.1 to 5.6 ± 0.2. The percentage of macrophages from MAb GK1.5-treated mice that phagocytized yeasts (9%) was not different from that of control mice (9.3%). Thus, MAb GK1.5 did not alter either intracellular growth of *H. capsulatum* or phagocytosis.

DISCUSSION

In this study, we showed that selective and profound depletion of the L3T4$^+$ subpopulation of T lymphocytes in lymphoid organs of C57BL/6 mice is associated with poor inhibition of *H. capsulatum* yeast cell multiplication. This was evident both by the significantly increased numbers of *Histoplasma* CFU recovered from the spleens of treated mice as compared with infected controls and by the failure of mice given MAb GK1.5 to survive a sublethal inoculum. Although the importance of T lymphocytes as mediators of host resistance to *H. capsulatum* has been recognized for some time (13, 17), the present study demonstrates unequivocally that L3T4$^+$ cells are a critical determinant of an effective host response to this pathogen.

It was possible that the numbers of L3T4$^+$ cells detected by flow cytometry were low either because the in vivo-injected MAb GK1.5 blocked the subsequent binding of fluorescein-labeled MAb GK1.5 or because of antigenic modulation rather than target cell depletion. Both of these possibilities seem remote for the following reasons. First, if the L3T4$^+$ antigen was masked by unlabeled MAb GK1.5, then these cells should be detectable by fluorescein-conjugated anti-rat IgG. However, the mean percentage of cells (± SEM) from treated mice that stained with fluorescein-conjugated anti-rat IgG (1.0 ± 0.3%) was similar to that of controls (0.6 ± 0.2%). Second, both L3T4$^+$ and Lyt-2$^+$ cells bear Thy-1.2, and the sum of the two subpopulations should approximate the number of Thy-1.2$^+$ cells. If antigenic modulation of L3T4 had occurred, the number of Thy-1.2$^+$ cells should exceed greatly the sum of L3T4$^+$ and Lyt-2$^+$ cells. This was not the case, thus suggesting that elimination of L3T4$^+$ cells was not caused merely by a loss of this antigen from the surface.

Of interest was the fact that granulomatous inflammation was present in the spleens of mice administered anti-L3T4 antibody and that the inflammatory response appeared similar to that found in spleens of infected controls. This histopathological picture contrasts with a recent report that MAb GK1.5 treatment dramatically suppresses granulomatous inflammation in livers of mice infected with *Schistosoma mansoni* (9). The reasons for the discrepant results between these two models remain unclear. In our system, it is conceivable that the small number of remaining L3T4$^+$ cells in MAb GK1.5-treated animals might have been sufficient to generate inflammatory mediators. On the other hand, the finding that mice depleted of L3T4$^+$ cells can mount a vigorous granulomatous inflammatory response to *H. capsulatum* suggests that this granulomatous process is not strictly dependent on L3T4$^+$ T cells. For example, colony-stimulating factors which may play a role in mobilizing mononuclear phagocytes from bone marrow to sites invaded by pathogenic microbes are produced by a wide variety of cells including T cells, fibroblasts, macrophages, and endothelial cells (18). Thus, release of such factors by host cells other than L3T4$^+$ cells may promote the granulomatous inflammatory response.

Several mechanisms should be considered to explain why MAb GK1.5 impairs host defense. First, since analogs of L3T4 are expressed by rat and human macrophages (8), this antibody may directly impair the biological functions of murine macrophages. However, there were no differences in the capacity of macrophages from normal mice or antibody-treated mice to function as antigen-presenting cells, to secrete IL-1 in response to lipopolysaccharide, or to phagocytose *H. capsulatum* yeasts. Others have shown that macrophages from mice are not lysed in vitro by the anti-L3T4 IgM MAb, RL-172.4, although this antibody is highly efficient in eliminating L3T4$^+$ T cells in vitro (14), and that peritoneal macrophages from mice do not bear the L3T4 antigen (3). Furthermore, the ability of splenic macrophages from mice treated with MAb GK1.5 to induce proliferation of *Leishmania major*-reactive T cells is identical to that of cells from mice given saline (14). It seems highly unlikely, therefore, that treatment with MAb GK1.5 influences macrophage function.

Second, because intracellular replication of *H. capsulatum* yeasts can be inhibited only by activated macrophages and not by resting cells (19), depletion of L3T4$^+$ cells may eliminate a source for lymphokines that can active macrophages. Among the family of macrophage-activating factors, gamma interferon appears to be the principal mediator that arms murine macrophages to exert anti-*Histoplasma* activity (19, 20). In this regard, it is well documented that L3T4 cells are one of the primary sources of gamma interferon (11). Thus, the vital role played by L3T4$^+$ T cells in controlling infection with *H. capsulatum* may be explained by the absolute requirement for the presence of these cells as a source of macrophage-activating factors, particularly, gamma interferon.

Third, in our model of disseminated histoplasmosis there is activation of splenic suppressor cell activity during the first 4 weeks of infection. As animals recover from infection by 6 to 8 weeks, the spleen cells from these mice exert helper activity and previously weak skin test reactivities to *Histoplasma* antigens become vigorous (2, 12). Treatment of *H. capsulatum*-infected mice with MAb GK1.5 not only eliminated the chief source of cells that exert helper function (16) but also failed to abrogate suppressor cell activity. As a consequence, the shift of immunoregulatory function from dominant suppressor activity to expression of helper activity cannot be achieved. Thus, persistence of suppressor cell activity combined with a deficiency of help appears to impair

seriously the capacity of the host to restrict infection with *H. capsulatum*.

Injection of MAb GK1.5 into mice during the resolving phase of infection (4 to 6 weeks) was not accompanied by a significant increase in the recovery of yeast cells from spleens. Therefore, the effect of MAb GK1.5 appears to depend on the phase of the cell-mediated immune response during which treatment is initiated. More specifically, host defense mechanisms against *H. capsulatum* may be susceptible to alteration by MAb GK1.5 only when the cell-mediated response is developing rather than during the later stages of resolution. It is possible that reactivation of infection requires a longer period (>2 weeks) of immunodeficiency or that the elimination of L3T4+ cells alone is insufficient to cause reactivation.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-17339 and AI-23017 and grant BRSG 507 RR05408025 from the National Institutes of Health and in part by a grant from the University of Cincinnati Research Council. G. S. Deepe is the recipient of Research Career Development award K04 AI-00856 from the National Institute of Allergy and Infectious Diseases.

We thank Angela Birch Smith for excellent secretarial assistance.

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